THE INFLUENCE OF AUXINS AND METHANOLIC EXTRACTS OF *HIBISCUS ROSA-SINENSIS* L. ON ROOT PROMOTION OF ASEPTICALLY CULTURED STEM CUTTINGS OF *ERYTHROXYLON COCA* L.

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN HORTICULTURE

MAY 1980

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Introduction

Plant growth substances are important factors in root formation. One group of these substances--the auxins--are known to exert considerable influence on the rate and quality of root formation.

Highly disparate rooting responses between juvenile and mature forms of the same species and between different cultivars of the same species have led to the extraction of chemical factors that promote rooting. Previous studies conducted by this worker (unpublished) evaluated a number of factors including auxins, cytokinins, gibberellins, sucrose, vitamins, and mineral salts for root promoting activity. These factors were studied individually and in numerous combinations but satisfactory rooting was not obtained. The postulation that difficult-to-root plants lacked in quality and/or quantity some rooting cofactors was an attractive hypothesis for testing (44, 45).

Extracts from a red-flowered, easy-to-root red Hibiscus variety have demonstrated a root-promoting influence in the presence of auxins (98). Erythroxylon coca nodal segments as well as multiple shoots derived from shoot tip cultures have displayed a shy-rooting habit. Hence, the goal of this study was to evaluate chromatographed fractions of a Hibiscus rosa-sinensis L. extract for efficacy in the promotion of rooting in a woody shrub, Erythroxylon coca L. under aseptic conditions. The synergistic action between the extract and auxins was also studied.

Literature Review

Aseptic Method

Aseptic tissue culture methods involve the maintenance of plant parts (protoplasts, cells, tissues, organs, etc.) under sterile conditions. There are numerous examples in the literature regarding the use of tissue culture techniques. For example, tissue culture can beused to increased the rate of clonal multiplication (18, 38, 42, 70), produce plant by-products in culture (9, 58) and to study physiological processes (90, 94).

In some forms of aseptic culture, callus--a mass of undifferentiated cells--is formed. These latter cells may undergo differentiation to form shoots, roots and eventually complete plantlets.

In still other types of tissue culture work, plant parts may be maintained in their original forms, e.g. maintenance of cell cultures suspended in liquid media without attempts to induce differentiation. Some of these studies utilize the relative simplicity of aseptically maintained plant parts to facilitate their research. Some physiological and biochemical processes may be more easily examined under sterile conditions (37, 92).

Auxins

Auxins play a ubiquitous role in plant growth and development. They are known to exert considerable influence in fruit set, apical dominance, abscission, cambial activity, nutrient translocation and a host of other plant responses (76, 101).

Early studies detailed the efficacy of auxins in root promotion (12, 13). Today, auxins are used extensively to promote rooting (18). Indole-butyric acid (IBA) ranks as the most widely used auxins for root promotion. A synthetic auxin, IBA is largely unaffected by the plants' natural auxin-inactivating systems (102). IBA is generally applied basally to stimulate rooting; its limited mobility reduces any undesireable effects on non-target tissues in the plant (102). Naphthalene-acetic acid (NAA) is also used to stimulate rooting. Because NAA is more toxic than IBA, concentrations of the auxin must be carefully monitored to avoid plant injury (41).

Indole-acetic acid is a natural auxin compound. The close structural similarity of tryptophan and IAA, nutritional experiments with Rhizopus sinuis (88, 95), and other studies (32, 105) showed that tryptophan was the primary precursor of IAA. Various metabolic studies, including experiments utilizing radioactive labelling techniques were able to elucidate the various pathways from tryptophan to IAA: the indolepyruvic acid (80, 81), tryptamine (75, 89), indoleacetaldoxime (105) and tryptophol pathways (82).

IAA is subject to numerous modes of catabolism. It may be oxidized into oxindoles, acetophenones and indolaldehydes (20, 54, 88). The activity of IAA oxidase can be affected by any of a number of naturally occurring compounds (88). Studies regarding phenols have shown that monophenols tend to act as cofactors of IAA oxidase, while o- and p-dihydric phenols and polyphenols are IAA oxidase inhibitors (30, 77). The phenolic IAA oxidase inhibitors include low molecular weight compounds such as chlorogenic and protocatechuic acids (88) as well as some high molecular weight compounds such as those isolated from Pharbitis nil (106). The active site of one of these high molecular weight compounds is probably an ortho-dihydroxyphenol (93). Protector activity tends to diminish with the ageing of tissue (106).

IAA oxidation requires O_2, Mn^{2+} and a phenolic (63). Phenolics such as ferulic acid serve as IAA cofactors of oxidation (34). While monophenolics can promote IAA oxidase, o-diphenolics can inhibit the activity of IAA oxidase (35).

In addition to oxidation, IAA can also be bound to other molecules in a form that renders it inactive (57, 81).

Other Plant Growth Regulators in Relation to Rooting

Although auxins are the group of plant growth regulators most often associated with root promotion, the other groups are also factors of interest.

Gibberellins are a group of naturally-occurring plant growth regulators that are principally noted for their effects in stem elongation. Gibberellins have generally demonstrated negative effects on root initiation (41). Gibberellins may prevent cell division in mature tissue and consequently block the formation of root initials. Alternatively, this group of plant growth regulators may promote shoot growth at the expense of the root system which is nutritionally deprived (102). However, Hess and others (26, 27, 44) found that the easily rooted juvenile form of English ivy contains more gibberellin than the mature forms. This anomaly suggests that other substances or systems may be modifying the effect of gibberellic acid. The theory suggests that the effect of gibberellic acid may depend upon the irradiance under which the stock plants were grown (39).

Cytokinins are natural or synthetic compounds that stimulate cell division and differentiation. Auxin/cytokinin ratios affect meristematic differentiation. A low auxin/cytokinin ratio favored bud formation while a high auxin/cytokinin ratio favored the formation of root primordia (41, 102).

Inhibitors evoke varied rooting responses in plants. Decreased rooting in Eucalyptus grandis has been correlated to increased levels of inhibitory substances (74). Other inhibitors may promote root initiation. Roughly 5 times the amount of ABA was found in juvenile English ivy than in mature forms. It is possible that ABA serves to antagonize the GA-induced inhibition of rooting. This may explain the relative ease of rooting in juvenile English ivy, with its ordinarily inhibitory endogenous level of GA (49, 85, 86). Abscisic acid can stimulate rooting in some plants (11).

Ethylene, one of the simplest plant growth hormones, is noted for its effects in the promotion of roots. The biological effects of ethylene include the promotion of germination (102), the acceleration of fruit ripening (8, 67) and the regulation of sexual expression (87). Additionally, ethylene is a known promoter of rooting (17, 59). The gaseous nature of ethylene made it a difficult compound to handle in field studies. However, with the advent of ethylene-releasing compounds such as ethephon (102), it is being used more widely.

Rooting Cofactors

While auxins are capable of root-promotion in a large number of plants, some plants do not root readily, even in the presence of auxins (52). Went proposed the existence of a root-promoting substance, "rhizocaline," that is produced in the leaves and transported basipetally to the site where it promotes rooting (104). Certain grafting and girdling experiments also suggested the existence of naturally-occurring substances other than auxins which promote rooting (6, 36, 72, 92, 98, 99). In one of these studies, van Overbeek and Gregory noted that an easy-to-root red-flowered Hibiscus and a difficult-to-root white-flowered Hibiscus responded differently to basally applied IBA. The auxin greatly promoted rooting of the red variety, but had little or no effect on the white variety. Following observations that the white-flowered Hibiscus defoliated soon after the cuttings were taken, workers hypothesized that a root-promoting factor was synthesized in the leaves and transported basipetally to the site of action. In subsequent tests, defoliated red-flowered Hibiscus plants demonstrated poor rooting, even with IBA. Additionally, when cuttings of red-flowered Hibiscus were grafted onto the white-flowered variety, the rooting response of the combination with the white base to IBA application was excellent.

Hess (44, 45) examined methanolic extracts from easy- and difficult-to-root forms of Hibiscus rosa-sinensis L. and Hedera helix L. When these extracts were chromatographed in an 80 percent isopropanol

solvent system, Hess found four groups of growth substances that promoted root formation. Both qualitative and quantitative differences were detected between the extracts of different species, different varieties, and plants of different maturities. While each of the groups had root-promoting activity, they were most efficient in the presence of IAA. Hess named these groups rooting cofactors 1, 2, 3, and 4 according to their increasing distance from the chromatogram origin. He designated them "rooting cofactors" because he felt that they were extractible substances responsible for the relative ease of rooting of certain plants.

Rooting cofactors have been studied in many species including Camellia (84), Castanea (31, 100), Chrysanthemum (46, 91), Dahlia (4), Eucalyptus (74), Hedera (26, 27, 33, 37, 44, 45, 46, 47, 48, 51), Hibiscus (44, 45, 92), Juniperus (60, 61), Malus (1, 66, 78, 79), Pelargonium (53), Phaseolus (50, 53), Pinus (108), Phoenix (83), Portulaca (68), Pyrus (24, 78), Rhododendron (62), Salix (31, 55, 56), and Taxus (61).

Other Factors That Affect Rooting

A stem cutting that is supplied with growth regulators and rooting cofactors in adequate quantity and quality may still fail to root. Unsatisfactory rooting may be due to the lack of mineral or organic nutrients. Media formulations that are used in plant tissue cultures may be suitable for some species but not others (28). Mineral salts, particularly nitrates, calcium and boron, are important in root stimulation (14, 15, 29, 96, 103). Carbohydrates also affect root formation (25, 40). Girdling may stimulate rooting by causing an accumulation of carbohydrates above the girdle (6). However, growth regulators, cofactors and other substances may also accumulate.

The nature and condition of the stock plant can directly relate to the ease or difficulty of rooting. The age of the stock plant can have a striking influence on the ease of rooting. English ivy, Hedera helix L., is one of these plants. Juvenile English ivy cuttings root far more readily than their mature counterparts (44, 45). The ease of rooting of juvenile English ivy cuttings is related to endogenous levels of growth regulators and tissues (44).

Lighting conditions can affect rooting; etiolated cuttings often root more readily than others. Herman and Hess (43) observed that IBA-treated etiolated Hibiscus stems rooted more readily and had a slightly higher auxin content than nonetiolated ones. Etiolated Salix tissue is also higher in auxin than the nonetiolated portions (56).

The higher levels of auxin in etiolated tissue can be explained, in part, by the reduced photodestruction of IAA. Alternatively, enhanced rooting of etiolated cuttings may be due to the absence of photo-inactivation of another essential factor in a root promoting complex (92). Other studies have found that light grown plants contain higher levels of inhibitors than etiolated plants (21, 96). Still another theory suggests that light inhibits root initiation through a supraoptimal carbohydrate level in relation to the endogenous auxin content (40).

Materials and Methods

Methanolic tissue extracts obtained from Hibiscus rosa-sinensis plants, indole-acetic acid (IAA), indole-butyric acid (IBA) and naphthalene-acetic acid (NAA) were evaluated for root-promoting effects on Erythroxylon coca. The primary function of aseptic culture methods in this study was to facilitate an investigation of rooting response without the presence and possible influence of other agents such as fungi, bacteria, etc.

Erythroxylon coca, a member of the Erythroxylaceae family, is a shrub native to South America. The plant is 1 to 4.5 meters high with leaves that are pale beneath, 5 - 9 cm long, alternate, and transovalate or ovalate-lanceolate. Ivory flowers, about 1 cm in diameter, are clustered at leaf axils. The plants have a five-lobed calyx, five hypogynous petals, ten stamens joined at the base, and a three-celled ovary with three styles. The fruit is an oblong, unilocular drupe, about 1 cm in length (7, 65).

E. coca is widely cultivated in the highlands of South America, where its leaves are harvested for the tropane alkaloid, cocaine (7, 10). While synthetic compounds have replaced most of the clinical uses of cocaine, unique vasoconstrictory properties of cocaine make it a highly desirable anesthetic for some types of surgical procedures (2).

Hibiscus rosa-sinensis L. is a member of the Malvaceae family. Native of Asia, it is now widely cultivated in Hawaii as an ornamental. It is a shrub that can attain a height of more than six meters. The

bell-shaped flowers are about 10 cm in diameter and range from the most common red to yellow and magenta. Leaves are narrow to broad-ovate, 7 - 10 cm long, shiny, and coarse-toothed (73).

Tissues for extraction were obtained from a single red-flowered, easy-to-root Hibiscus rosa-sinensis L. plant. Fully expanded leaves with the incident nodal tissue were selected for extraction. 1.0 g of fresh tissue was lyophilized. The lyophilized tissue was extracted three times with 20 ml aliquots of absolute methanol at 0°C. Each extraction period was 30 minutes, with periodic agitation. The aliquots were combined, filtered and reduced in vacuo to dryness. The residue was taken up in 1 ml methanol and was streaked on 5 cm-wide strips of Whatmann No. 3 mm chromatographic paper (92). The chromatograms were equilibrated overnight before development in a descending manner in isopropanol and water (8:2, v/v). After the solvent front had advanced 30 cm, the chromatogram was removed and air dried. Each chromatogram was divided into strips corresponding to 0.1 R_F unit. Chromatogram segments above the origin were used as controls to test for any possible activity of the chromatographic solvents alone. Each strip was placed into a borosilicate culture tube containing 20 ml of the medium listed in Table 1. Teh tubes were gently agitated for 30 minutes. A hot water bath maintained the media in a liquid state udring this period. Subsequently, the strips were removed and the tubes autoclaved at 15 psi for 20 minutes. Treatments were replicated 15 times.

Table 1

Modified Murashige and Skoog Medium (71)

Component	mg/l
$CaCl_2 \cdot 2H_2O$	440
$CoCl_2 \cdot 6H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8
H_3BO_3	6.2
KH_2PO_4	170
KI	0.83
KNO_3	1900
$MgSO_4 \cdot 7H_2O$	370
$MnSO_4 \cdot 4H_2O$	223
$Na_2MoO_4 \cdot 2H_2O$	0.25
$Na_2 \cdot EDTA \cdot 2H_2O$	37.3
NH_4NO_3	1650
$ZnSO_4 \cdot 7H_2O$	8.6
Sucrose	30,000
Inositol	100
Thiamine · HCl	0.5
Pyridoxine · HCl	0.1
Glycine	2
Agar	8,000

pH adjusted to 5.7

Fifteen chromatograms were assayed before and after autoclaving to determine whether heat sterilization affected biological activity. The mung bean (Phaseolus aureus) bioassay was performed after the procedure outlined by Hess (44) and modified by Blazich and Heuser (5).

Stem pieces of Erythroxylon coca were surface sterilized in 10 percent Clorox solution with 0.1 percent Tween 20 for 15 minutes. After three rinses in sterile, distilled water, the stems were divided into 2 cm nodal segments and inserted into the prepared medium.

While previous experience with Erythroxylon coca in aseptic culture had indicated that auxins alone were ineffectual in root-promotion, IAA, IBA, and NAA were re-evaluated for root-promoting activity. Basal medium described in Table 1 was prepared and auxins were added to separate tubes at the rate of 0, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 mg/l. Medium was autoclaved at 15 psi for 20 minutes. Sterile E. coca nodal segments 2 cm in length were placed in the medium of each tube. Treatments were replicated 15 times. There was no rooting response in any of the treatments after four weeks, although shoots elongated and unfolded new leaves. No further rooting occurred after the four week period.

In order to determine an optimal concentration of each of the auxins, tubes were prepared as described above, with the inclusion of methanolic Hibiscus extract that was the equivalent of 1.0 g of fresh tissue per tube. The extracts were prepared as described previously, except that the dried extract was taken up and dissolved in 20.0 ml of

media containing 0, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, or 50.0 mg/l of IAA, IBA or NAA. The 20 ml portion was dispensed into a culture tube and was autoclaved at 15 psi for 20 minutes. E. coca stem segments were maintained under cool-white fluorescent lights for 16 hrs. per day at 25 ± 2°C. After 4 weeks, the number of roots per tube was recorded.

The auxin levels that had evoked the greatest rooting response were selected to be used in combination with a 0.1 R_F unit strip of the chromatographed Hibiscus methanolic extracts. In this manner, the entire chromatogram was tested in 0.1 segments in the presence of an optimal level of auxin. Rooting response was recorded after 4 weeks.

Results and Discussion

While auxins are generally regarded as the premier group of growth regulators for root promotion, three auxins--indole-acetic acid (IAA), indole-butyric acid (IBA), and naphthalene-acetic acid (NAA)--in concentrations of 0 - 50 mg/l produced absolutely no root-promoting response in E. coca. This auxin inefficacy has been noted in a number of plants. In the case of Hibiscus, this has been found to occur in only some varieties (36, 98); additionally, juvenile forms of some plants such as English ivy (Hedera helix) may be much more responsive to auxins than the mature forms (44, 45). Clearly, in some shy rooting plants one or more factors aside from auxins are required to stimulate rooting.

Unsatisfactory rooting may be due to the lack of rooting hormones, cofactors, or nutritional factors in suitable quantity or quality (44, 45, 97). Poor rooting could also be attributed to certain anatomical features such as the presence of a ring of sclerenchyma or the absence of preformed root initials (3, 22). Alternatively, the presence of substances that inhibit rooting might require leaching or inactivation before rooting can occur (23).

Failure to elicit rooting in the presence of auxins alone necessitated the formulation of medium containing IAA, IBA, and NAA in concentrations varying from 0 - 50 mg/l plus a crude methanolic extract obtained from Hibiscus. Figures 1, 2, and 3 show the rooting response of E. coca to IAA, IBA, and NAA, respectively, in concentra-

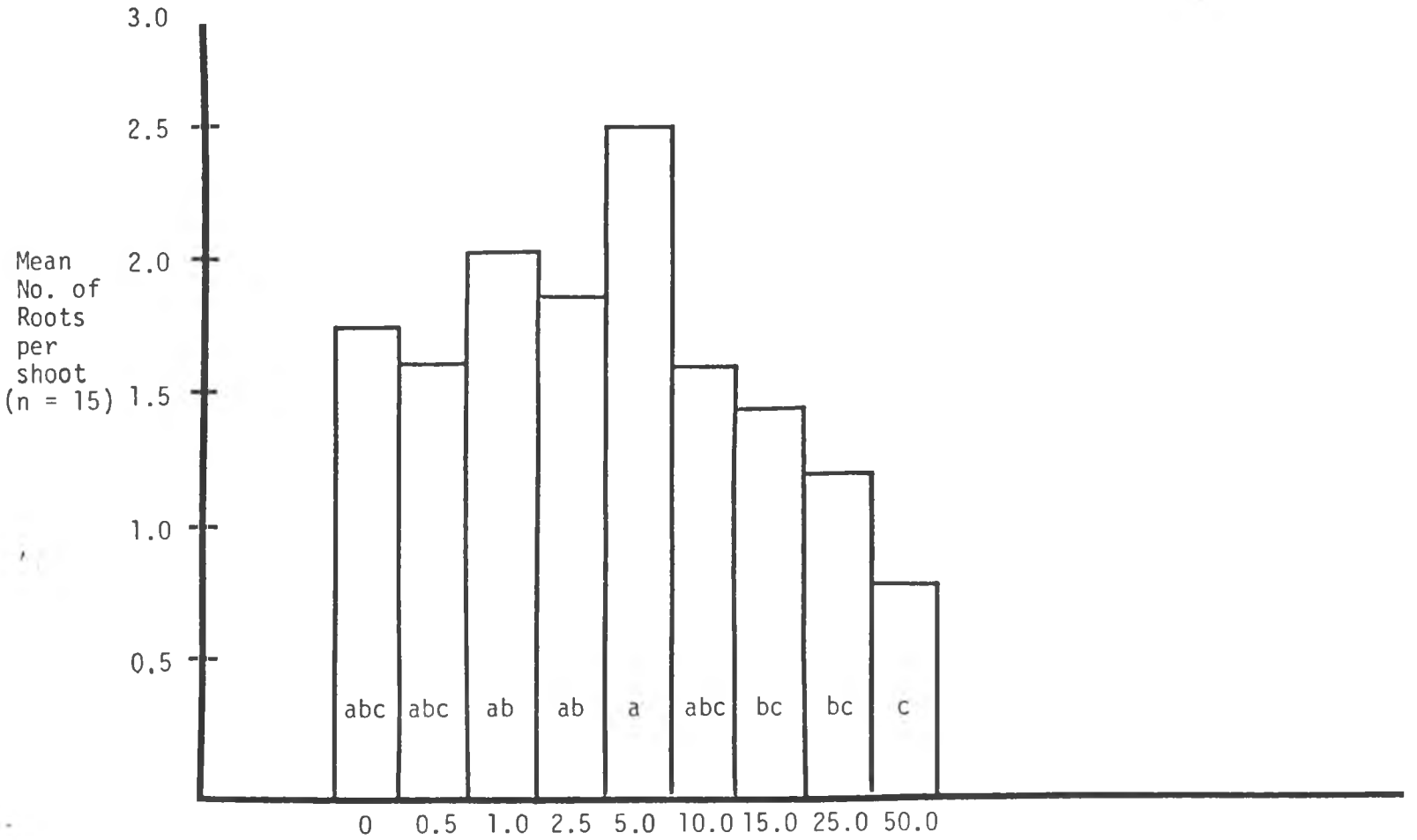

Figure 1. Rooting response of E. coca nodal stem segments to IAA concentration in the presence of methanolic Hibiscus extracts. Treatment means designated by the same letter are not significantly different at the 5% level, using Duncan's Multiple Range Test.

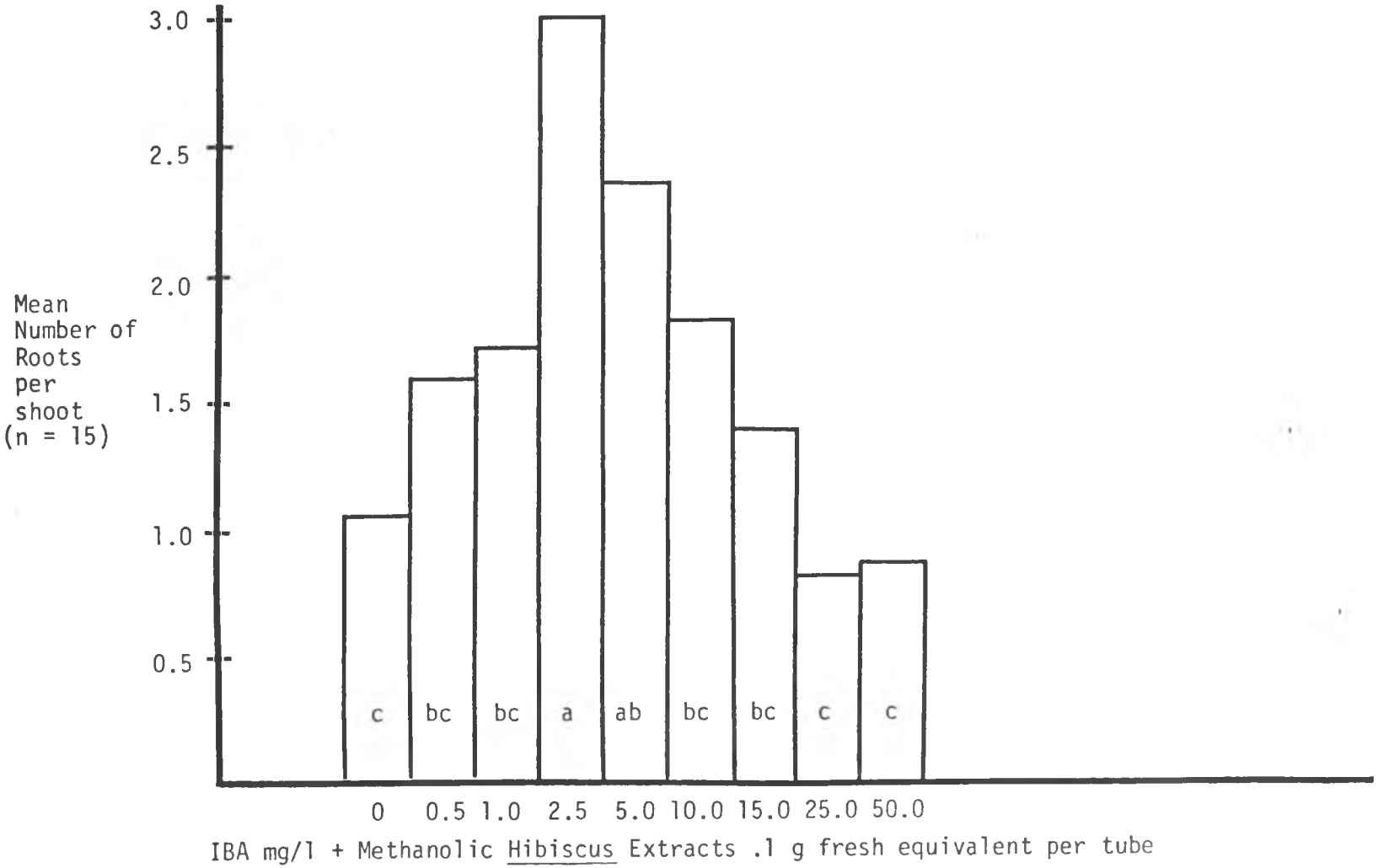

Figure 2. Rooting response of E. coca stem segments to IBA concentration in the presence of methanolic Hibiscus extracts. Treatment means designated by the same letter are not significantly different at the 5% level, using Duncan's Multiple Range Test.

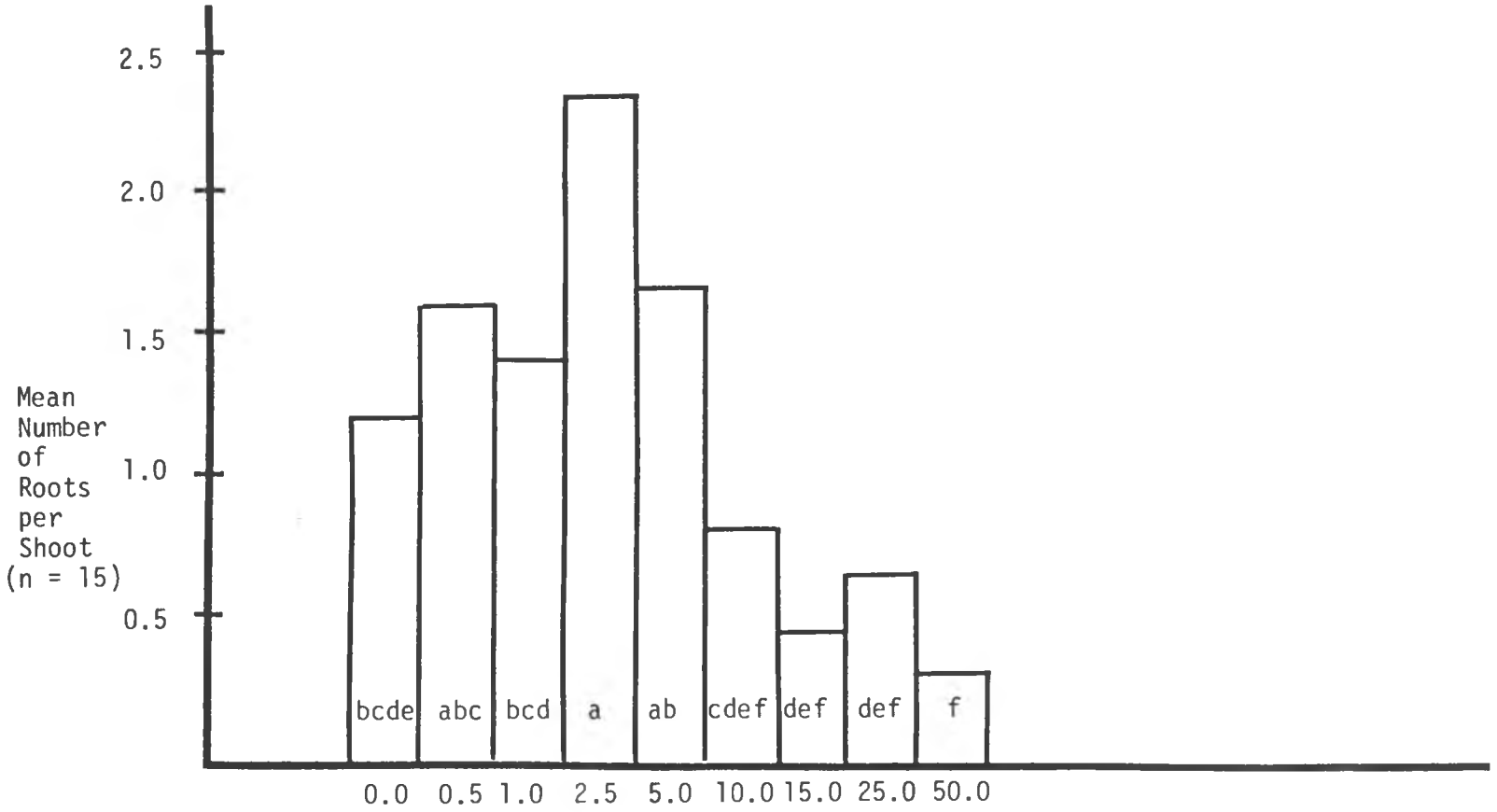

Figure 3. Rooting response of E. coca nodal stem segments to NAA concentration in the presence of methanolic Hibiscus extracts. Treatment means designated by the same letter are not significantly different at the 5% level, using Duncan's Multiple Range Test.

tions ranging from 0 to 50 mg/l.

While not significantly different from all other treatments at the five percent confidence level, root-promotion was superior when IAA, IBA, and NAA were present in concentrations of 5.0, 2.5, and 2.5 mg/l, respectively. These concentrations were selected for use in an evaluation of the effect of both auxins and chromatographed Hibiscus extracts on rooting.

Chromatographed fractions of methanolic Hibiscus extracts were bioassayed with the mung bean (Phaseolus aureus) rooting test to determine if the extract was thermolabile. Table 2 lists the mean rooting response of 10-day-old mung bean cuttings to chromatographed fractions of Hibiscus extracts in the presence of 1 mg/l IAA. There was essentially no difference in rooting response that could be attributed to the heat sterilization procedure. Three areas of the chromatograms were active in root promotion--these were at R_F units 0.2-0.3, 0.5-0.6 and 0.7-0.8. These R_F values correspond to cofactors 2, 3, and 4, respectively (44). Assays of the cofactor levels with the mung bean rooting test have showed the presence of cofactors 1, 2, 3, and 4 (44) and cofactors 1, 2, and 4 (91) in red Hibiscus extracts. Studies have demonstrated cofactors differences between red and white flowered varieties of Hibiscus. There are also differences between the red Hibiscus used in this experiment and other red Hibiscus plants used in other studies (44, 91). The absence of cofactor 1 in the red Hibiscus used in this study may be a varietal

TABLE 2. Mean number of roots per mung bean (*Phaseolus aureus*) cutting in response to chromatographed *Hibiscus* extracts before and after autoclaving.[1]

R_F units	Before autoclaving	After autoclaving
0 - 0.1	13.73 a	14.27 a
0.1 - 0.2	1.00 a	0.73 a
0.2 - 0.3	22.47 a	23.07 a
0.3 - 0.4	12.73 a	12.33 a
0.4 - 0.5	13.13 a	14.27 a
0.5 - 0.6	26.20 a	25.13 a
0.6 - 0.7	16.60 a	15.27 a
0.7 - 0.8	31.20 a	29.93 a
0.8 - 0.9	14.60 a	15.80 a
0.9 - 1.0	2.47 a	2.60 a

[1] Within each chromatogram section, means followed by the same letter are not significantly different at the 5% confidence level.

difference or it may be due to growth of the plant under different environmental conditions.

Figure 4 shows the rooting response of E. coca stem cuttings to methanolic Hibiscus extracts alone and in the presence of the optimal levels of the three different auxins. While the magnitude of the E. coca rooting response to Hibiscus extracts alone was largest in R_F unit 0.7-0.8, separation of treatment means by Duncan's multiple range test indicated no significant difference at the 5% confidence level between that site and many other sites on the chromatogram.

In all four treatments, 1) extract alone, 2) extract plus IAA, 3) extract plus IBA and 4) extract plus NAA, two sites--R_F 0.1-0.2 and 0.9-1.0 produced no rooting response.

With the addition of 5.0 mg/l IAA, the chromatographically fractionated Hibiscus extracts were highly active in root-promotion at sites 0.5-0.6 and 0.7-0.8 of the chromatogram. There was no significant rooting promotion among the chromatographic fractions with the addition of 2.5 mg/l IBA. In the Hibiscus extract plus 2.5 mg/l NAA treatments, there were no significant root promoter sites. In summary, when rooting responses were compared between chromatographed fractions of Hibiscus extract alone, or Hibiscus extract plus a single auxin treatment, only two strips in the IAA treatment showed any significant promoter activity.

The R_F locations of these sites of root-promotion correspond

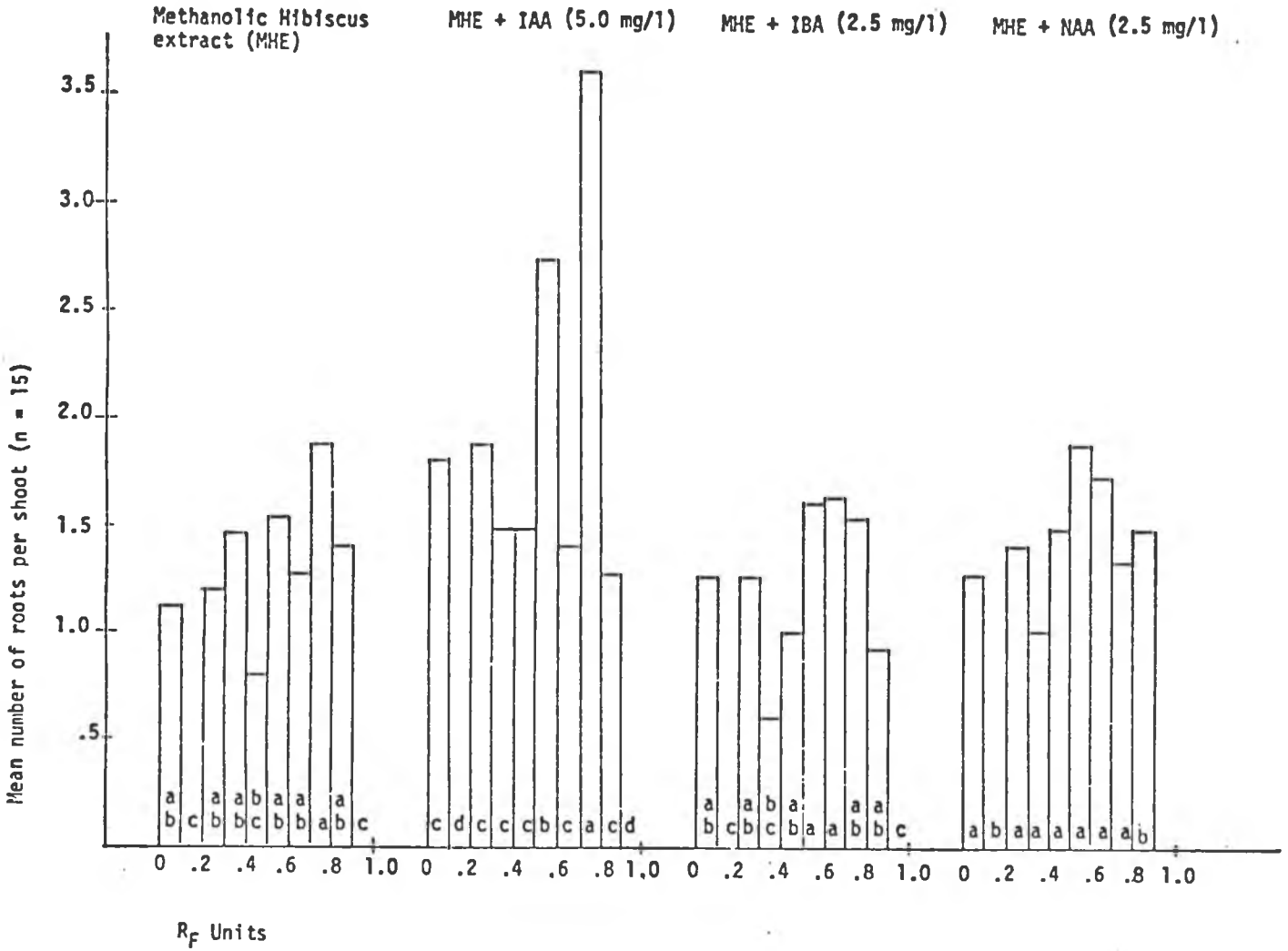

Figure 4. Rooting response of E. coca nodal segments to chromatographically fractionated Hibiscus methanolic extracts, IAA, IBA and NAA. Within sets, means which have the same letter are not significantly different from one another at the 5% level.

to cofactors 3 and 4 (44). Hess has attributed part of the biological activity of cofactor 3 to isochlorogenic acid (47). Catechol, and other phenolic compounds have been found to be active in root-promotion (19, 46). Other workers have indicated that some naturally occurring phenolic compounds may protect IAA from destruction, and consequently increase the pool of IAA that is available for root-promotion (107). The nature of the protection mechanism may be that certain phenolic substances act as inhibitors of the IAA oxidase system (88). Support of this proposal comes from the recognition that such an auxin-protector would have little or no effect upon NAA--an auxin that is not destroyed by the IAA-oxidase system (20). Hackett's work with English ivy shoot apices failed to show any synergism between NAA and catechol (37).

Instead of acting as auxin-protectors, phenolic substances may demonstrate synergism by forming an auxin-phenol complex that is more active than the separate components in the promotion of rooting (64). A root-promoting auxin-phenol complex has been isolated from hardwood pear cuttings (24). However, other work with English ivy found NAA to be an active root promoter in the absence of added phenols (37). In this study, the chromatogram segment corresponding to cofactor 3 reacted synergistically with only IAA. It cannot be determined in the scope of this experiment if the root-promoting activity is due to inhibition of the IAA-oxidase system or the synergism of an auxin-phenol complex.

Cofactor 4 has been identified as a mixture of lipoidal oxygenated terpenoids. The instability of purified samples have hampered attempts to further characterize the compounds (51). While it has been demonstrated that several auxins can bind naturally-occurring lipids, there is no evidence that links these interactions with biological activity (57).

Table 3 and Figure 5 present a comparison of the rooting response of different auxins at the same R_f site on the chromatogram. When data were subjected to analysis of variance. a significant root-promotion effect was found in the 0.8-0.9 site of the chromatogram. Following mean separation by Duncan's multiple range test, the IAA plus Hibiscus extract was found to be significantly different from the treatment mean for the Hibiscus extract alone in chromatogram sections 0.5-0.6 and 0.8-0.9.

The role of the rooting cofactors in root promotion is still controversial. A number of studies failed to establish correlation between cofactor level and rooting response. In a study of the factors influencing root initiation in easy- and difficult-to-root chrysanthemum, Stolz reported that although total carbohydrate level was well-correlated with root initiation, cofactor levels were not (91). Biran and Halevy found that endogenous inhibitor levels were responsible for the poor rooting response of a Dahlia; they reported no differences in the auxin or cofactor levels of easy- and difficult-to-root cultivars (4). Other studies have also reported poor correla-

TABLE 3. Mean number of roots per E. coca shoot in response to chromatographed Hibiscus extracts and auxins. (n = 15)

R_F Unit	Methanolic Hibiscus Extract (MHE)	MHE + IAA 5.0 mg/l	MHE + IBA 2.5 mg/l	MHE + NAA 2.5 mg/l	F-ratio
0 - 0.1	1.13	1.8	1.27	1.27	< 1
0.1 - 0.2	0	0	0	0	< 1
0.2 - 0.3	1.2	1.87	1.27	1.40	< 1
0.3 - 0.4	1.4	1.4	0.6	1.0	1.63 n.s.
0.4 - 0.5	0.8	1.47	1.0	0.8	< 1
0.5 - 0.6	1.53	2.73	1.60	1.87	2.56 n.s.
0.6 - 0.7	1.27	1.40	1.53	1.73	< 1
0.7 - 0.8	1.80	3.6	1.47	1.33	14.68 **
0.8 - 0.9	1.40	1.27	0.93	1.47	< 1
0.9 - 1.0	0	0	0	0	< 1

$\hat{F}_{(.05)} = 2.83$

$\hat{F}_{(.01)} = 4.29$

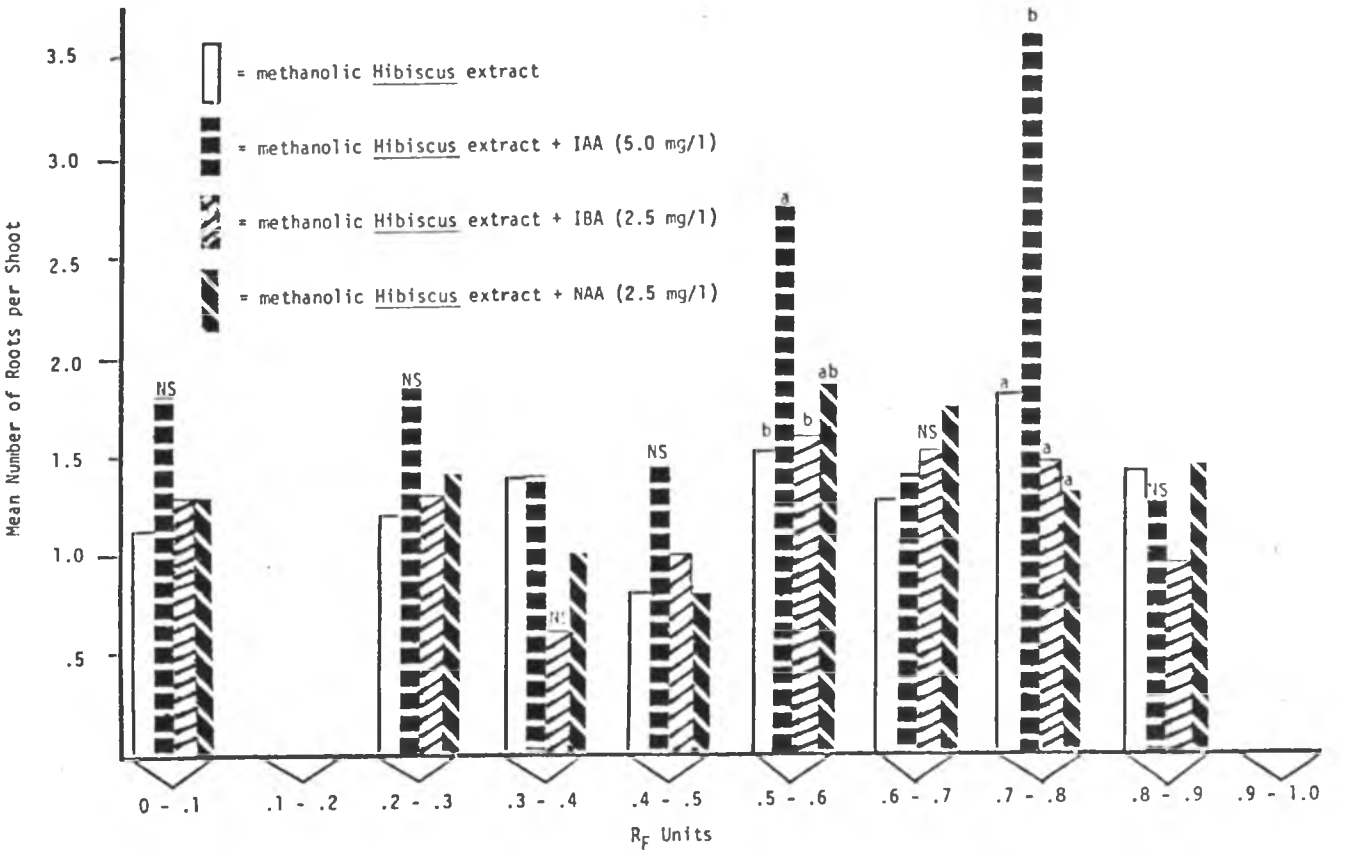

Figure 5. Root Promotion. Rooting response of E. coca stem segments to chromatographically fractionated Hibiscus methanolic extracts and IAA, IBA and NAA. Means designated by the same letter are not significantly different at the 5% level.

tion between cofactors and rooting (61, 84). In this experiment, the mung bean (Phaseolus aureus) bioassay indicated the presence of cofactors 2, 3, and 4, as determined by chromatographic position. However, E. coca shoots indicated the presence of only cofactors 3 and 4. In addition to variable cofactor extracts from different plants there are also variable responses of different species to these extracts. A plant extract may contain rooting inhibitors in addition to rooting cofactors. However, the presence of significant levels of inhibitory substances in difficult-to-root cuttings does not rule out the influence of cofactors in rooting. Their presence can reduce but may not necessarily eliminate the root-promoting effects of the cofactors.

Previous workers have evaluated the rooting response of E. coca shoots to chlorogenic acid and a number of phenolic compounds (unpublished). These substances were tested in combination with only one type of auxin--NAA. The results of this study suggest that the work be duplicated using IAA instead of NAA.

While Hess determined that isochlorogenic acid is a major component of cofactor 3, no positive identification has been made of cofactor 4 beyond recognition of its lipoidal nature. The precise chemical nature of cofactors 1 and 2 has not been determined. In the absence of good root promoters, crude extracts obtained from easy-to-root plants may be acceptable in spite of the undefined chemical nature of the extracts. In orchid tissue culture work, coconut water

and other undefined natural mixtures have long been used (69). Ideally, one would prefer positive identification of those substances that are the active principles for root promotion. If identification is difficult, and the use of a natural extract unfeasible, it would be desireable to identify certain substances that resemble the cofactors, with high specific activity in root promotion.

It has been proposed that plants may be classified according to the deficiencies that impede their rooting (4). The first group of plants are those which already possess auxin, cofactors and other native substances in sufficient quantity and quality for good rooting. When cuttings from these plants are maintained under the proper conditions, rooting occurs. The second group has adequate amounts of cofactors, but is deficient in auxin. The third group has sufficient auxin, but lacks ample amounts of the cofactors. The fourth group is deficient in both auxins and cofactors. While the E. coca shoots responded positively to the inclusion of methanolic Hibiscus extracts in the medium, the most striking levels of rooting occurred in response to both IAA and the extract. E. coca is apparently a member of the fourth group of plants--those requiring both auxins and cofactors for root promotion.

SUMMARY

1. Indole-acetic acid, indole-butyric acid and naphthalene acetic acid in concentration of 0 - 50 mg/l had no root promotion effect on *E. coca* stem segments.
2. While the addition of chromatographed *Hibiscus* extracts did promote rooting, the most significant increases occurred in the presence of R_F units 0.5 - 0.6 or 0.7 - 0.8 of the chromatographed extract and 5.0 mg/l IAA.
3. A combination of IBA or NAA at 2.5 mg/l and the *Hibiscus* extract was not more effective in root promotion than the extract alone.

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